CARDIOVASCULAR DIAGNOSTICS INC

TAS™ ECT™ Test Card

Kit Containing 25 ECT Test Cards

For *in vitro* diagnostic use only

Humanitarian Device: Authorized by Federal Law for use in determining the anticoagulant effect of recombinant hirudin (r-hirudin) during cardiopulmonary bypass (CPB) in patients who have heparin-induced thrombocytopenia (HIT). The effectiveness of this device for this use has not been demonstrated.

Federal law restricts this device for sale and distribution to, or on the order of, a physician or to a clinical laboratory. Use is restricted to, by, or on the order of a physician.

Intended Use

The TAS™ (Thrombolytic Assessment System) Ecarin Clotting Time (ECT) Test Card is to be used with the TAS Analyzer and is intended to determine the anticoagulant effect of r-hirudin during cardiopulmonary bypass in patients who have heparin-induced thrombocytopenia.

The TAS ECT (the TAS ECT Test Card together with the TAS Analyzer) is suited for professional use in decentralized areas of testing near the site of patient care as well as for use in the more traditional clinical laboratory.

Monitoring with the TAS ECT is indicated for persons who have been identified with and/or confirmed as a high risk for HIT, and require high dose anticoagulation with recombinant hirudin for a scheduled or emergency CPB procedure.

Contraindications

TAS ECT is contraindicated for patients on coumadin therapy with an INR > 4.5, patients with > 25% hemolysis, patients with > 30% hemodilution, patients with > 0.5 U/ml concentration of unfractionated heparin (UFH), and patients with less than 30% prothrombin activity or 15 mg/dl fibrinogen concentration. Patients on thrombolytic therapy should not be monitored with the TAS ECT. The use of acid citrate blood collection tubes is also contraindicated with the TAS ECT.

Summary and Explanation

Ecarin, a protein prothrombin activator from *Echis carinatus* venom (E.C. 3.4.99.27), was isolated by Komalik et al. in 1969.[1] Ecarin causes coagulation of citrated whole blood (CWB) or citrated plasma (CP) by the calcium-independent activation of prothrombin. Ecarin has been characterized by Morita et al.[2] and by Komaflik and Blombäck[3] as a single chain glycoprotein with a molecular weight of 55-60 KDaltons which exerts metalloproteinase activity inhibited by EDTA, glutathione, cysteine, and mercaptoethanol. Common serine proteinase inhibitors such as diisopropyl-fluorophosphate (DFP), soybean trypsin inhibitor (SBTI), ovomucoid and aprotinin do not inactivate ecarin.

Ecarin catalyzes the hydrolytic cleavage of the 323Arg-324Ile bond in the human prothrombin molecule, whereby thrombin activity is generated without the release of any zymogen fragment. This form of active prothrombin has been termed meizothrombin and is inhibited by r-hirudin, PEG-hirudin, and low molecular weight synthetic thrombin inhibitors such as argatroban, but not efficiently by the heparin-ATIII complex.

Ecarin has been used to develop a sensitive analytical method for the determination of thrombin inhibition by the antithrombin drug r-hirudin.[4,5] The application for this test is to monitor the anticoagulant effect induced in a patient receiving r-hirudin during cardiopulmonary bypass.[6,7]

The TAS ECT Test Card is based on the method of Nowak and Bucha.[4] The TAS system is designed to eliminate many of the variables such as transport and handling that are encountered with other coagulation methods.

Principle

The TAS ECT Test Card is a one-stage, two-step test that measures the clotting time of a blood sample. The patient's blood sample is first diluted with pooled normal human plasma. Then, the diluted sample is added to the dry reagent on the prewarmed ECT Test Card. By using ecarin to activate prothrombin, the coagulation cascade (activation of factors V – XII) is bypassed and a deficiency in one or more of these factors will not be reflected in the result.

Reagent

Components	Storage	Stability
Ecarin calcium chloride, buffers, stabilizers, and paramagnetic iron oxide particles.	2–8°C (36–46°F)	Unopened–24 months or until expiration date
	or,	
	20–25°C (68–77°F)	Unopened—2 weeks

WARNING: Exposure of the test cards at any time to magnetic objects or fields (for example, an MRI instrument) may corrupt the encoded information and prevent the analyzer from starting the test.

CAUTION: Any pouches not kept refrigerated should be dated and not be used beyond this 2-week period. Pouches should not be repeatedly warmed and returned to the refrigerator. Once the pouch is opened, the card must be used within 15 minutes.

POTENTIAL ADVERSE EFFECTS OF THE DEVICE ON HEALTH: Inaccurate information on the coagulation status of the patient poses a significant risk to the patient during CPB since both over-treatment and under-treatment can have fatal consequences (bleeding in the patient and clots in the CPB system, respectively). Possible risks of using the TAS ECT Test Card could include underestimation or overestimation of the level of hirudin in the patient. Currently, an antidote for hirudin is unavailable, and the clinical data available are insufficient to establish the probabilities or incidence of erroneous medical judgements and their clinical outcomes.

POTENTIAL BIOHAZARD: All components of this product are of nonhuman origin, and as such, should not contain HBV, HCV, or HIV. Nevertheless, since absence of infectious agents cannot be proven, all samples (e.g., patient blood) and products (e.g., standard and control plasma) obtained from human blood should always be handled with due care, observing the precautions recommended for biohazardous material.[6,7]

Specimen Collection and Preparation

The TAS ECT Test Card is to be used with citrated whole blood collected and processed according to recognized standards for the handling of blood specimens for coagulation studies.[7] The blood should be added to either 109 or 129 mmol (3.2% or 3.8%) of the dihydrate form of sodium citrate in a proportion of nine (9) parts whole blood to one (1) part anticoagulant. The blood should be mixed well with the anticoagulant immediately upon collection.

- The citrated whole blood sample must be diluted one to one (1:1) with pooled normal human plasma prior to testing.* Precise pipetting of the sample is important.
- Using a 100 µl pipette, dispense 100 µl of the citrated whole blood into a polypropylene plastic tube.

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* SARP (Helena) and FACT (George King) plasmas were used as the pooled normal human plasma for all the ECT studies performed at CVDI. Use of any other pooled normal human plasma may give different results.

- Using the same 100 µl pipette with a new pipette tip, add 100 µl of pooled normal human plasma to the plastic tube containing 100 µl of citrated whole blood. Mix the sample.
- Using a sample transfer device capable of delivering approximately 30-35 µl, transfer 30-35 µl diluted whole blood to the sample test well when prompted by the analyzer.
- Diluted whole blood should be tested within 15 minutes of collection to avoid *ex vivo* changes that may affect the clotting time results.

Materials Required but not Provided

- TAS Analyzer
- TAS Operator Manual
- Pooled normal human plasma
- Blood sampling materials such as venipuncture needles, syringes, alcohol swabs, vacutainer tubes containing sodium citrate
- Sample transfer devices (pipettes with tips or droppers) capable of delivering approximately 30 - 35µl
- Two levels of quality control plasma available from Cardiovascular Diagnostics, Inc.
- 100 µl pipette with tips
- Polypropylene tubes

Directions for Use

1. Equilibrate test cards at room temperature (20 – 25°C) before removing from the foil pouch. No further preparation of the TAS ECT Test Card is necessary prior to beginning the test.

   CAUTION: The test card must be used within 15 minutes after the pouch is opened. Pouches of cards should not be repeatedly warmed and returned to the refrigerator.
2. Remove the test card from its foil pouch and hold it so that the full name is right side up, facing you.
3. Pass the test card firmly and steadily through the card reader.

   The analyzer interprets the encoded information on the test card and displays prompts for each step of the procedure.
4. When prompted, place the test card in the analyzer, and allow to warm.

   CAUTION: Do not leave the test card in the analyzer longer than 15 minutes before applying the sample. Prolonged warming of the card can affect the performance of the test.
5. When prompted, add approximately 30-35 µl of the 1:1 diluted sample into the sample well on the test card.
6. At the end of the test, confirm that the test was performed with the analyzer set to the appropriate sample type. (Sample type is displayed along with the result at the end of each test.)
7. When the card is removed from the analyzer at the end of each test, ensure that the entire reaction chamber was filled with sample. If an inadequate amount of sample was added to the card, repeat the test, using a fresh card.
8. The TAS Analyzer will display the ECT results within 1 to 12 minutes, depending on how long it takes the sample to clot. **CVDI provides no recommendation on dosing of r-hirudin.** The user should dose the patient with r-hirudin in view of the actual medical situation, local experience with the drug, and the pertinent labeling instructions for r-hirudin.
9. Dispose of the test card and other contaminated items in a manner approved for biohazardous materials.

Procedural Notes

- The TAS Analyzer is preset to provide a constant reaction temperature of 37 ± 3°C, and will automatically prewarm the test card before prompting the user to apply the sample drop. All other necessary parameters are magnetically encoded on each test card. Please refer to the TAS Analyzer Operator Manual for details of instrument use
- Operate the TAS Analyzer only at ambient temperatures between 18 to 32°C.
- To maintain a fully charged battery, leave the unit plugged into its power supply, which is in turn plugged into an AC outlet. Leave the power switch in the "OFF" position while storing the analyzer.
- The Operator Identification Code and the Quality Control Lockout are optional features. Refer to the Operator Manual if either of these features has been enabled.
- Ensure that the sealed pouch containing a test card has reached room temperature and that the TAS Analyzer is either plugged into an appropriate wall outlet or has a sufficiently charged battery.
- Collect and dilute blood in a 1:1 ratio with pooled normal human plasma* as described in the Specimen Collection and Preparation Section.

Quality Control

Calibration: No user calibration is necessary with the TAS ECT Test Card. Calibration of the TAS Analyzer was performed at CARDIOVASCULAR DIAGNOSTICS, INC.

Routine Quality Control Procedures: Prior to each CPB use, the operator should verify that the date and time displayed on the analyzer are accurate. Reset if necessary. (See the TAS Operator Manual for instructions.) This step ensures that the operator will be warned of any attempt to use an expired test card.

The microprocessor in the TAS Analyzer automatically monitors the parameters necessary for accurate testing. If the TAS Analyzer detects an error during the performance of the test, it will display an error message. (See the TAS Analyzer Operator Manual for details and an explanation of error messages.)

At the end of the test, the operator should confirm that the test was performed with the analyzer set to the appropriate sample type. The sample type is displayed along with the result at the end of the test.

When the card is removed from the analyzer at the end of each test, the operator should be sure that the entire reaction chamber (all of the gray area) was filled with sample. If an insufficient amount of sample was added to the card, the test should be repeated, using a fresh card. Dispose of the test card and other contaminated items in a manner appropriate for biohazardous material.

The test card should not be left in the analyzer for longer than 15 minutes before application of the sample. Prolonged warming of the card may affect the performance of the reagent.

Functional Quality Control Testing: Quality control of the total system should be monitored by testing two levels of quality control plasma. Instructions from the manufacturer for reconstitution of these materials must be strictly followed. Both normal and abnormal levels should be run prior to CPB.

Results

The TAS ECT result is reported in seconds and is displayed on the TAS Analyzer screen at the end of the test.

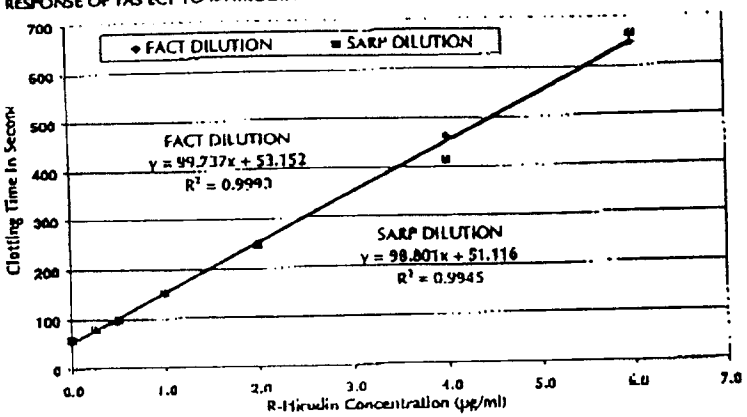
Expected Values

Samples from 120 normal individuals (72 females and 48 males ranging in age from 20 to 63 with a median age of 38) were tested using the TAS Analyzer and TAS ECT Test Cards. Citrated whole blood was diluted 1:1 with pooled normal human plasma. The range (mean ± 2 S.D.) obtained was 41.6 to 55.3 seconds. This range is given for illustrative purposes only. Results reported as <25 seconds or >700 seconds should be verified by repeat testing.

Fresh citrated whole blood was diluted 30% with phosphate buffered saline (PBS). The PBS/blood mixture was supplemented with various concentrations of r-hirudin and the aliquots were diluted 1:1 with either FACT or SARP plasma, and (five) 5 replicate measurements were performed on TAS-ECT tests (30µl/test).

Results are shown in the graph below. Both FACT and SARP normal pooled plasmas yielded equivalent sensitivity to r-hirudin.

COMPARISON OF TWO DIFFERENT POOLED NORMAL HUMAN PLASMAS ON THE RESPONSE OF TAS ECT TO R-HIRUDIN



Specific Performance Characteristics

Analytical sensitivity

Studies show that the TAS ECT Test Card reagent is sensitive to r-hirudin to 100 ng/mL or 0.1 µg/mL.

Precision

Studies were performed using the TAS Analyzer and TAS ECT Test Cards to evaluate test precision. 4.0 µg/ml was the highest level of r-hirudin used in precision studies, and precision at higher levels of r-hirudin has not been determined. Two levels (0 and 4.0 µg/ml) of r-hirudin were analyzed with citrated whole blood. Aliquots of these samples were then diluted 1:1 with pooled normal human plasma (All precision studies were performed using FACT plasma from George King). The following results were produced.

Within Run Precision
(N = 30 each) These results include instrument to instrument and dilutional precision. Samples for these results were CWB with a 30% PBS dilution.

	0.0 µg/ml r-hirudin:	4.0 µg/ml r-hirudin:
Mean (sec)	55.1	443.4
Std. Dev. (sec)	2.8	27.4
C.V. (%)	5.0	6.2

Operator to Operator Precision
(N = 30 each) These results include instrument to instrument and dilutional precision. Samples for these results were CWB with a 30% PBS dilution.

	0.0 µg/ml r-hirudin:			4.0 µg/ml r-hirudin:		
Operator	1	2	3	1	2	3
Mean (sec)	49.8	50.7	52.2	405.3	396.0	419.2
Std. Dev. (sec)	3.1	2.7	2.6	30.1	22.8	23.1
CV (%)	6.2	5.3	5.0	7.4	5.8	5.5

Day to Day Variation of ECT Test Card
(N = 10 control samples per day for 20 days) These results include Instrument to instrument precision.

	Normal Control	Range		Abnormal Control	Range	
		min	max		min	max
Mean (sec)	52.9	50.8	54.7	154.0	146.1	159.9
Tot. Precision (S.D.)	2.2			9.2		
Tot. CV (%)	4.1			6.0		

Day to Day Variation of ECT Test Card
(N = 5 samples per day for 20 days) Samples for these results were CWB with a 30% PBS dilution.

	0.0 µg/ml r-hirudin	Range		4.0 µg/ml r-hirudin	Range	
		min	max		min	max
Mean (sec)	55.9	49.0	60.6	439.4	393.0	529.0
Tot. Precision (S.D.)	4.1			35.5		
Total CV (%)	7.0			6.7		

Day-to-Day Precision – Citrated whole blood samples: Day-to-Day precision was also evaluated (N= 5 samples per day for 20 days) using TAS ECT results compiled from the various predinical studies performed at Cardiovascular Diagnostics, Inc. These results include Lot-to-lot (total of 6 different ECT lots were used), instrument-to-instrument (at least 5 different TAS analyzers were used each day), and day-to-day precision. Results also include imprecision due to use of multiple donors. These data were analyzed according to "Evaluation of Precision Performance of Clinical Chemistry Devices; Approved Guideline,NCCLS publication EP5-A, Volume 19 Number 2." Total precision standard deviation was 4.1 for citrated whole blood samples with 0.0 µg/ml r-hirudin and 35.5 for citrated whole blood samples with 4.0 µg/ml r-hirudin. Since these data were obtained from multiple TAS analyzers (5), instrument variation was also included in these estimates.

Lot to Lot Precision and Response

R-hirudin (0.0 or 2.0 µg/mL) was added to an undiluted citrated whole blood sample from a normal donor. This sample was evaluated on three lots of TAS ECT Test Cards. Forty replicates were performed for each test card lot.

Lot #	r-hirudin	Mean (sec)	SD (sec)	CV (%)
1	0.0	48.6	2.4	5.0
	2.0	428.6	19.9	4.7
2	0.0	49.3	2.0	4.1
	2.0	397.6	27.8	7.0
3	0.0	48.6	2.0	4.0
	2.0	434.1	25.2	5.8

Results of all the precision testing indicate that the major contribution to imprecision is test-to-test card variation and not instrument-to-instrument, donor-to-donor, or operator-to-operator.

Interferences

Interference studies were performed on 3.2% citrated whole blood samples or 3.8% citrated plasma samples. These samples were diluted 30% with phosphate buffered saline (PBS, pH 7.3, 50 mM phosphate, 150 mM NaCl) and supplemented with r-hirudin at 0.0 and 4.0 µg/ml. Patient samples containing coumadin (INR ≤ 4.5) should not affect the results of the ECT test. CP samples were used to determine the effect of factor deficencies on test performance. Less than 30% activity of normal prothrombin (Factor II) will cause prolongation of the ECT. Fibrinogen levels of 15 to 1000 mg/dl have no significant effect on the ECT test results.

CWB samples were used for the following interference tests. Acidified citrate cannot be used to obtain samples for this test. Hematocrits to 64%, lipids to 15 mg/ml, nitroglycerin to 1000 µg/ml, aprotinin to 1000 KIU/ml, dextran to 5 mg/ml, protamine to 100 µg/ml, and sample temperature of 4 to 37°C have no effect on TAS ECT test performance. Unfractionated heparin (UFH) levels below 0.5 U/ml have no effect on test performance. Hemolysis of 25% or less should not effect test performance; however presence of hemolysis is often an indicator of poor specimen quality. Hemodilution greater than 30% causes a statistically significant increase in TAS ECT test results. Plasminogen levels <20% affect ECT test results.

References

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CARDIOVASCULAR DIAGNOSTICS INC

Cardiovascular Diagnostics, Inc.

Thrombolytic Assessment System (TAS)
Ecarin Clotting Time Test

Patient Information

HUMANITARIAN DEVICE:

Authorized by Federal Law for use in determining the anticoagulant effect of recombinant hirudin (r-hirudin) during cardiopulmonary bypass (CPB) in patients who have heparin-induced thrombocytopenia (HIT). The effectiveness of this device for this use has not been demonstrated.

Federal law restricts this device for sale and distribution to, or on the order of, a physician or to a clinical laboratory. Use is restricted to, by, or on the order of a physician.

GENERAL INFORMATION AND PROCEDURES:

The CVDI TAS Ecarin Clotting Time test (ECT) is intended to be used to determine the anticoagulant effect of recombinant hirudin (r-hirudin) during cardiopulmonary bypass (CPB) in patients who have heparin-induced thrombocytopenia (HIT).

The physician uses the results of the TAS ECT test to determine whether the patient has been sufficiently anticoagulated during CPB. This affords the physician the opportunity to adjust the dosage of r-hirudin accordingly.

POSSIBLE BENEFITS AND RISKS OF USING THE TAS ECT TEST:

The potential benefit of using the TAS ECT test is that r-hirudin therapy can be guided using TAS ECT test results. Possible risks include:

- under- or overestimation of r-hirudin levels in patients leading to bleeding or clotting episodes, and
- inaccurate information on coagulation status in patients.

ALTERNATIVE PRACTICES AND PROCEDURES

Currently, the performance of *in vitro* diagnostic coagulation procedures, such as the activated partial thromboplastin time (aPTT), activated clotting time (ACT) and the prothrombin time (PT) tests has not been established for monitoring r-hirudin at the levels necessary during CPB.

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